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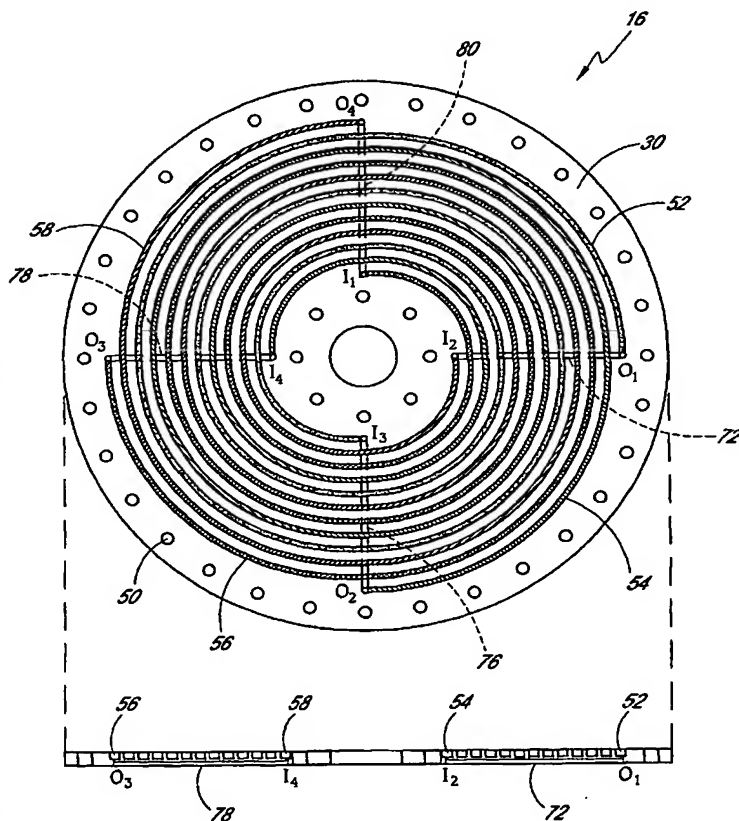
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(54) Title: **METHOD AND APPARATUS FOR COUNTERCONCURRENT CHROMATOGRAPHY**

SEPARATION DISK WITH 4 SPIRAL CHANNELS



(57) Abstract: A countercurrent chromatography apparatus includes a plurality of plates, at least one plate (16) having first and second interleaved spiral flow channels (52, 54, 56, 58) therein. Each spiral flow channels (52, 54, 56, 58) has a first end (I₁, I₂, I₃, I₄) near the central axis and a second ends (O₁, O₂, O₃, O₄) near the periphery. The outlet of the first channel (O₁) is connected to the inlet of the second channel (I₂) by a connecting channel (72). Septa may be provided between the plates to connect the spiral channels of one plate to the spiral channels of the next plate.

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METHOD AND APPARATUS FOR COUNTERCURRENT CHROMATOGRAPHY

Background of the Invention

Field of the Invention

[0001] The invention relates to countercurrent chromatography systems, and more particularly to an improved column design for use in countercurrent chromatography.

Description of the Related Art

[0002] Chromatography is a separation process that is achieved by distributing the substances to be separated between a mobile phase and a stationary phase. Those substances distributed preferentially in the moving phase pass through the chromatographic system faster than those that are distributed preferentially in the stationary phase. As a consequence, the substances are eluted from the column in inverse order of their distribution coefficients with respect to the stationary phase.

[0003] Chromatography is widely used for the separation, identification, and determination of the chemical components in complex mixture. Chromatographic separation can be utilized to separate gases, volatile substances, nonvolatile material, and polymeric material including biological substances.

[0004] The performance of countercurrent chromatography systems depends largely on the amount of stationary phase retained in the column, which determines both the resolving power of the solute peaks and the sample loading capacity. Numerous countercurrent chromatography systems have been developed to optimize the retention of the stationary phase of a sample in the column. The maximum attainable retention level tends to fall sharply with the application of higher flow rates of the mobile phase, resulting in loss of peak resolution. Consequently, the applicable flow rate has become one of the major limiting factors in countercurrent chromatography.

[0005] Some countercurrent chromatography systems utilize a complex hydrodynamic motion in two solvent phases within a column comprising a rotating coiled

tube. If, for example, a horizontally mounted coil is filled with water and is rotated around its own axis, any object, either heavier or lighter than the water present in the column will tend to move toward one end of the coil. This end is then called the "head" and the other end, the "tail" of the coil. When the coil is filled with two immiscible solvent phases, the rotation establishes a hydrodynamic equilibrium between the two solvent phases, where the two phases are distributed in each turn at a given volume ratio (equilibrium volume ratio) turn and any excess of either phase remains at the respective tail of the coil for each solvent.

[0006] When one of the solvents is added to the coil from the tail end and is further eluted from the coil from its head end, the hydrodynamic equilibrium tends to maintain the original equilibrium volume ratio of the two phases in the coil and thereby a certain volume of the other phase is permanently retained in the coil while the two phases are undergoing vigorous agitation with rotation of the coil. As a result, the sample solutes present in one phase and introduced locally at the inlet of the coil are subjected to an efficient partition process between the two phases and are chromatographically separated according to their partition coefficients.

[0007] In some cases, countercurrent chromatography utilizes a multi-layer coil as a separation column to produce a high efficiency separation with relatively favorable retention of the stationary phase in many solvent systems. Thus, countercurrent chromatography has been employed to achieve efficient extraction of a sample solution under relatively high flow rates. Previous column designs have relied on the use of a helical coil of tubing. U.S. Patent No. 4,430,216, hereby incorporated by reference in its entirety, describes a preparative countercurrent chromatography utilizing a multiple layer coiled column. The coiled column design includes a length of plastic tubing wound around a coil holder to form multiple layers of the coil. Although this system works reasonably well for some solvents, these systems often fail to retain a satisfactory amount of the stationary phase for highly viscous, low interfacial solvent systems such as polymer phase systems, which are useful for the separation of macromolecules and particulates. In addition, the coiled tubing configuration is difficult to assemble, and connecting the ends of neighboring spiral tubing is rather difficult.

Summary of the Invention

[0008] In one embodiment, the invention comprises a countercurrent chromatography apparatus comprising a plurality of plates, wherein at least one of the plurality of plates comprises at least first and second interleaved spiral flow channels formed therein. Each of the spiral flow channels includes a first end and a second end, wherein the first ends are closer to a central axis of the plate than the second ends, and wherein the second end of the first spiral flow channel is in fluid communication with the first end of the second spiral flow channel. The apparatus may additionally comprise a plurality of septa positioned between pairs of the plates, wherein at least some of the plurality of septa comprise a hole which is positioned to establish a fluid connection between a second end of a spiral flow channel in one of the plurality of plates and a first end of a spiral flow channel in a second of the plurality of plates.

[0009] In another embodiment, a countercurrent chromatography apparatus comprises a column assembly, wherein the column assembly comprises a plurality of coupled separation disks, and wherein each of the separation disks comprises at least two interleaved spiral flow channels.

[0010] In another embodiment, a plate for use in countercurrent chromatography comprises a first surface, a second opposed surface, and a plurality of interleaved spiral flow channels, each having an inner end and an outer end. At least one flow path connects an outer end of at least one of the spiral flow channels to an inner end of a different one of the interleaved spiral flow channels.

[0011] Methods of performing chromatography are also provided. In one embodiment, the method comprises routing fluid in a first groove from an inner end to an outer end of a first spiral flow path, routing the fluid in a second groove from the outer end of the first spiral flow path to an inner end of a third groove, and routing fluid in the third groove from an inner end to an outer end of a second spiral flow path.

Brief Description of the Drawings

[0012] FIGURE 1 is a perspective view of a column assembly for use in high speed countercurrent chromatography.

[0013] FIGURE 2 is an exploded view of the column assembly depicted in Figure 1.

[0014] FIGURE 3 is a top view of a separation disk with a single spiral channel.

[0015] FIGURE 4A is a top view of a separation disk having a plurality of spiral channels.

[0016] FIGURE 4B is a cross section of the separation disk of Figure 4A.

[0017] FIGURE 4C is a bottom view of a separation disk as illustrated in Figure 4A.

[0018] FIGURE 5 is a top view of a septum.

[0019] FIGURE 6 is a top view of an upper flange and gear.

[0020] FIGURE 7 is a bottom view of a lower flange.

Detailed Description of the Preferred Embodiment

[0021] Embodiments of the invention will now be described with reference to the accompanying Figures, wherein like numerals refer to like elements throughout. The terminology used in the description presented herein is not intended to be interpreted in any limited or restrictive manner, simply because it is being utilized in conjunction with a detailed description of certain specific embodiments of the invention. Furthermore, embodiments of the invention may include several novel features, no single one of which is solely responsible for its desirable attributes or which is essential to practicing the inventions herein described.

[0022] Although embodiments of the invention have various applications, many advantageous embodiments of the present invention are directed to an improved column for use in countercurrent chromatography. Applicable chromatography techniques include those using synchronous planetary motion such as X-type, J-type, and I-type chromatography. The apparatus and methods described herein are especially advantageous when applied to high-speed countercurrent chromatography (HSCCC) with high flow rates. The column design may also be employed in large column applications for industrial-scale separations of samples by mounting the column assembly on a slowly rotating horizontal shaft. Some aspects of the invention are based, in part, on the surprising discovery that the retention of the stationary phase is improved if the

configuration of the column used in counter current chromatography is modified from coiled tubing to a series of grooved plates (also referred to herein as separation disks) forming a plurality of interleaved spiral channels. The centrifugal force gradient produced by the spiral pitch in the separation disks helps to more efficiently distribute the heavier phase in the periphery and the lighter phase in the proximal portion of the column.

[0023] This centrifugal force effect on a sample is enhanced by increasing the pitch of the spiral, and a spiral column assembly prepared by simply winding tubing into a flat spiral configuration like thread on a spool provides only a limited spiral pitch. In accordance with these observations, a column for use in high speed counter-current chromatography has been developed having interleaving, grooved separation disks with multiple flow channels instead of coiled tubing. The use of a column having interleaving, grooved separation disks has many advantages over the prior art. First, a column possessing a plurality of interleaving, grooved separation disks with multiple flow channels provides a greater spiral pitch than previous column designs, facilitating the movement of a fluid sample radially outward at a faster rate than with previous designs, thereby providing a more efficient separation of the two phases of a fluid sample. Finally, the column design of the present invention obviates the shortcomings of earlier column designs inasmuch as it is easier to manufacture.

[0024] Figure 1 illustrates a perspective view of one embodiment of a spiral column assembly 10 made in accordance with some aspects of the invention. A hollow column holder shaft 20 is provided with a coaxial planetary gear 22 coupled to an upper flange 12. The planetary gear 22 is configured to be coupled to an identical stationary gear, which is mounted on the central axis of a centrifuge (not illustrated). The gear arrangement provides a synchronous planetary motion of the column with respect to the centrifuge. As a result, a hydrodynamic equilibrium of the solvent system is established such that the two phases within the column are separated along the length of the column. Notably, either phase becomes usable as the mobile phase. As is shown in more detail in Figure 2, the column is made from a coupled plurality of adjacent plates. Advantageous plate embodiments are illustrated in more detail below with reference to Figures 2-4.

[0025] The column assembly 10 may be mounted on a rotary frame of any suitable multilayer coil centrifuge as is described in U.S. Patent No. 4,430,216, previously incorporated by reference in its entirety. In one embodiment, the column assembly 10 is

removeably mounted to a mulilayer coil centrifuge manufactured by P.C. Inc. (Potomac, MD) and retentively engaged by detachable bearing blocks secured to the centrifuge by means of fastening screws. The bearing blocks also serve to counterbalance the column assembly.

[0026] Still with reference to Figure 1, a pair of flow tubes 24, 26 are led through a center hole in the column holder shaft 20 downward and out of the column to exit the complete centrifuge apparatus at a center hole in an upper plate of the whole centrifuge assembly where the flow tubes 24, 26 may be tightly fixed with a pair of clamps (see, for example, the routing shown in U.S. Patent 4,430,216). Advantageously, these flow tubes 24, 26 may protected with a sheath of flexible tubing such as Tygon® (Norton Company, Worcester, MA) to prevent direct contact with metal parts in the centrifuge assembly.

[0027] Figure 2 is an exploded view of the components of the column assembly illustrated in Figure 1. The column assembly 10 comprises an upper flange 12 having a gear 22 and a lower flange 14. Disposed between the upper flange 12 and the lower flange 14 is a plurality of coupled separation disks 16. Alternating between each of the plurality of separation disks 16 is a septum 18. The separation disks 16 can be constructed from stainless steel or a plastic such as a PTFE, high density polyethylene, or any other suitable polymer. Advantageously, the separation disk 16 may have a diameter of between 1 cm and 30 cm, and a thickness of between 0.5 and 20 mm. In one embodiment that has been found suitable, the separation disk 16 has a diameter of about 17.5 cm and a thickness of about 4 mm. Preferably, the septum 18 is constructed of PTFE (e.g. Teflon®, E.I. Du Pont, Wilmington, DE). One of skill in the art would appreciate that the septum 18 can be constructed from any number of suitable non-reactive materials. The construction of the separation disks 16 and septa 18 are described in additional detail below with reference to Figures 3, 4A, and 4B.

[0028] Turning now to Figure 3, a first embodiment of a separation disk 16 having a first surface 30 and a second opposed surface 32 is illustrated. The separation disk 16 includes an inner edge 40 and an outer edge 42. The separation disk 16 comprises a single spiral flow channel 44 carved, etched, or molded on the surface of the first side 30 of the separation disk 16. The spiral flow channel 44 has an inlet end 46 and an outlet end 48 with fluid flow typically traveling along the path of the spiral channel 44 from the

inlet end 46 to the outlet end 48. Advantageously, the spiral channel 44 of one separation disk 16 is serially connected to the spiral channel of another separation disk by stacking multiple separation disks adjacent to one another with a septum separating each pair. Preferably, the outlet end 48 of the channel 44 connects to the inlet end of the channel on the next adjacent disk. To accomplish this, the bottom of the outlet end 48 of the channel 44 includes a hole that is connected to a channel 49 grooved or molded into the other side of the disk with a hole (which may be about 1 mm diameter) that extends through the thickness of the disk. The channel 49 on the other side of the disk extends radially inward until it is substantially aligned with the inlet end 46 of the spiral channel 44. This inner end of the channel 49 is adjacent to a hole in the septum that connects to the inlet end of the spiral channel on the next adjacent disk.

[0029] To hold the disks together in making the whole column, the separation disk 16 advantageously includes a plurality of screw holes 50 at regular intervals at both the inner 40 and outer 42 edges of the separation disk 16. In some suitable embodiments, the screw holes 50 are positioned approximately 10 degrees for the outer edge 42 and 45 degrees for the inner edge 40. Similar holes are also made in both the septa and the flanges as will be described in greater detail with reference to Figures 5,6, and 7.

[0030] In a preferred embodiment, multiple interleaved spiral flow channels are incorporated symmetrically around the center of a separation disk 16 so that the spiral pitch is increased as compared to the spiral pitch of a single spiral channel such as is shown in Figure 3. Figure 4A is a top view of the first surface 30 of a separation disk 16 having a plurality of interleaved spiral channels. Figure 4B is a cross section of the disk of Figure 4A, and Figure 4C is a top plan view of the disk of Figures 4A and 4B. As illustrated in these Figures, the separation disk 16 has four separate spiral channels 52, 54, 56, and 58, respectively. However, it will be appreciated that the number of spiral channels can vary. Each channel may be between 0.25 mm and 10 mm wide. Preferably, the width of each channel may be between 0.5 mm and 7 mm wide, with 3 mm having been found suitable in one embodiment. The depth of each channel can likewise vary. Preferably, the depth of each channel is between 0.1 mm and 5 mm, with 2 mm having been found suitable in one embodiment. Depending on the size of the disk, each channel may have a length of between about 250 mm and 5 m. In one advantageous embodiment, the length of each channel is approximately 1 m. It will be appreciated, however, that the

length of each channel can vary. Each groove or channel is separated from the next groove or channel by a ridge 60, which may measure approximately 1 mm in width for 3 mm wide channels.

[0031] Still with reference to Figure 4A, each channel has an inner end denoted I_1 , I_2 , I_3 , and I_4 respectively in Figure 4A. The channels each begin at their inner ends and spiral around to their outer ends denoted O_1 , O_2 , O_3 , and O_4 respectively in Figure 4. In the embodiment of Figure 3B, each channel forms 3.25 spiral turns so that the outer end of a given channel is at the same angular location relative to the inner end of the next channel. Thus, as shown in Figure 4A, O_1 is at the same angular orientation as I_2 , O_2 is at the same angular orientation as I_3 , etc.

[0032] Connection between an outlet end of one spiral channel and an inlet end of the next spiral channel is made by another channel formed on the second opposed surface 32 of the separation disk 16 as illustrated with dashed lines on Figure 4A and also on Figure 4B, which shows a top plan view of the second opposed surface 32. Except for the inlet end of the first channel (I_1), each end of the channel has a hole 70 (approximately 1 mm in diameter) through the disk 16 which communicates with the end of a channel on the opposite surface 32 of the disk 16.

[0033] Referring now to Figures 4A, 4B, and 5, when a sample solution is introduced into the column assembly, fluid enters the inner end I_1 of the first channel and travels in the direction of the spiral to the outer end O_1 of the first channel. At this point, the fluid travels through hole 70 to the end of the linear channel 72 on the other side of the disk, thereby providing a flow path for the sample to travel from the outer end O_1 of the first channel to the radial location of the inner end I_2 of the next channel. From there the fluid flows through another hole extending between the inner end of the linear channel 72 and the inner end I_2 of the second spiral channel. The fluid then travels around to the outer end O_2 of the second channel, where it passes through a hole to radially extending channel 78 on the other side of the disk, from where it travels to the location of the inner end I_3 of the third channel. This process is repeated through the third and fourth channels until the fluid ends up at the outer end O_4 of the fourth channel. At this point, the fluid passes through another hole at the outer end O_4 of the fourth channel and through radial channel 80 to the position of I_1 where the flow started. However, no hole through the disk to the inner end I_1 of the first channel is provided. Rather, a hole 84 in the septum 18 is

positioned to coincide with the location of the inner end of the flow channel 80 on one disk and with the inner end of the first channel on the next adjacent disk. Thus, in a series of adjacent disks as shown in Figure 1, fluid passes from the end of the fourth channel on one disk to the beginning of the first channel on the next disk.

[0034] In operation, the pitch of each spiral increases markedly as compared to the pitch of a single spiral channel. For example, when a separation disk includes four spiral channels, the pitch can become as large as 16 mm (three times that of the single spiral channel).

[0035] One embodiment of the flanges which are placed on the top and bottom of the stack of disks of Figure 1 is shown in Figures 6 and 7. The upper flange 12 (Figure 6) is equipped with a gear 22 which engages with a stationary gear on the HSCCC centrifuge. Figure 7 depicts the lower flange 14 which has two screw holes 90, 92 positioned substantially 90 degrees apart for tightly fixing the column assembly against the column holder shaft (0.9 inch diameter). Both the upper and lower flanges 12, 14 each have an inlet/outlet hole 94 which fits to an adapter with a screw thread. They also have a set of screw holes 60 located at regular intervals around the outer and inner edges as in the separation disks and Teflon septa. In preferred embodiments, the screw holes 60 are positioned approximately 10 degrees apart for the outer edge and 45 degrees apart for the inner edge.

[0036] It has been observed that the use of a rectangular spiral channel embedded in a solid separation disk as described above enhances the retention of the stationary phase for viscous, low interfacial tension two-phase solvent systems. Accordingly, in one embodiment, a column having a separation disk comprising at least one rectangular spiral channel embedded in the disk is provided. The rectangular spiral channel configuration has a number of advantages over the prior art. For example, the rectangular spiral channel is useful for separating biopolymers such as proteins, DNA, RNA, polysaccharides, and cell particles. Additionally, this channel design ensures reliable retention of the stationary phase for polar or low interfacial tension solvent systems such as the 1-butanol/water system to separate bioactive compounds including peptides. Similarly, the rectangular design provides an improved stationary phase retention against emulsification.

EXAMPLE

[0037] A suitable highly viscous, low interfacial two-phase solvent system is thoroughly equilibrated in a separatory funnel at room temperature and the two phases are separated before use. The sample solution is prepared by dissolving the sample in a proper volume (e.g. 1-5 ml) of the upper and/or lower phase of the solvent system. The spiral column assembly is first entirely filled with the stationary phase (upper or lower phase), followed by sample injection through the sample port. The apparatus is rotated at 800 rpm while the mobile phase is eluted through the column at a desired flow rate. The separation may be repeated by changing the direction of the revolution and/or elution mode (i.e., head to tail and tail to head), although it is expected that the best result would be obtained by eluting the lower phase from the internal terminal toward the external terminal of the spiral channel at tail to head elution mode or the upper phase from the opposite direction in the head to tail mode.

[0038] The effluent from the outlet of the column is continuously monitored through a uv detector and fractionated into test tubes for later analysis.

[0039] In accordance with the foregoing, certain embodiments of the invention provide an improved column design for use in high speed countercurrent chromatography, which increases the retention of the stationary phase and increases efficiency of the chromatographic system. It will be appreciated, however, that no matter how detailed the foregoing appears in text, the invention can be practiced in many ways. As is also stated above, it should be noted that the use of particular terminology when describing certain features or aspects of the invention should not be taken to imply that the terminology is being re-defined herein to be restricted to including any specific characteristics of the features or aspects of the invention with which that terminology is associated. The scope of the invention should therefore be construed in accordance with the appended claims and any equivalents thereof.

WHAT IS CLAIMED IS:

1. A countercurrent chromatography apparatus comprising:
a plurality of plates, wherein at least one of said plurality of plates comprises at least first and second interleaved spiral flow channels formed therein, wherein each of said spiral flow channels includes a first end and a second end, wherein said first ends are closer to a central axis of said plate than said second ends, and wherein the second end of said first spiral flow channel is in fluid communication with the first end of the second spiral flow channel.
2. The apparatus of Claim 1, additionally comprising a plurality of septa positioned between pairs of said plates, wherein at least some of said plurality of septa comprise a hole which is positioned to establish a fluid connection between a second end of a spiral flow channel in one of said plurality of plates and a first end of a spiral flow channel in a second of said plurality of plates.
3. The apparatus of Claim 1, additionally comprising upper and lower plates, and wherein at least one of said upper plate and said lower plate comprises a gear.
4. A plate for use in countercurrent chromatography comprising:
a first surface;
a second opposed surface;
a plurality of interleaved spiral flow channels, each having an inner end and an outer end; and
at least one flow path connecting an outer end of at least one of said spiral flow channels to an inner end of a different one of said interleaved spiral flow channels.
5. The plate of Claim 4, wherein said flow channels comprise grooves formed in said first surface.
6. The plate of Claim 5, wherein said flow path comprises a groove formed in said second surface.
7. The plate of Claim 6, wherein said groove is substantially linear.
8. The plate of Claim 4, wherein said plurality of spiral flow channels have a substantially rectangular cross section.

9. The plate of Claim 5, comprising four interleaved spiral grooves in said first surface.

10. The plate of Claim 6, comprising four interleaved spiral grooves in said first surface and four radially extending grooves in said second surface.

11. A countercurrent chromatography apparatus comprising a column assembly, wherein the column assembly comprises a plurality of coupled separation disks, and wherein each of said separation disks comprises at least two interleaved spiral flow channels.

12. The apparatus of Claim 11, wherein outer ends of each spiral flow channel are in fluid communication with inner ends of different spiral flow channels.

13. A method of performing countercurrent chromatography comprising:

providing a test sample;

introducing said sample into a column assembly comprising:

an upper flange having a gear;

a lower flange;

a plurality of adjacent separation disks disposed between said upper and said lower flanges, wherein each of said plurality of separation disks comprises at least one spiral flow channel having a first end and a second end, and wherein the second end of the at least one spiral flow channel of one of said plurality of separation disks is in fluid communication with the first end of the spiral flow channel of another of said plurality of separation disks; and

a plurality of septa positioned between separation disks, wherein said plurality of septa comprise a hole positioned such that fluid can travel from the second end of the spiral flow channel of one of said plurality of separation disks through said hole of said plurality of septa to the first end of the spiral flow channel of another of said plurality of separation disks;

rotating said column assembly about an axis, whereby said rotation causes said sample to pass through said at least one spiral channel of said plurality of interleaving separation disks; and

eluting fluid from said column.

14. A method of manufacturing a column assembly for use in high speed countercurrent chromatography comprising:

(a) grooving a first spiral channel having a first end and a second end into a first surface of a disk;

(b) grooving at least a second spiral grooved channel interleaved with said first spiral channel and having a first end and a second end into the first surface of a disk;

(c) connecting the second end of the first spiral grooved channel with a radial flow channel on a second surface of the disk; wherein said radial flow channel is in fluid communication with the first end of said second spiral grooved channel on the first surface of said disk;

(d) coupling a plurality of disks formed according to steps (a), (b), and (c) via septa that comprise at least one hole.

15. The method of Claim 14, wherein said disk is formed from plastic.

16. The method of Claim 15, wherein said plastic is high density polyethylene.

17. The method of Claim 14, wherein said disk is stainless steel.

18. A method of performing chromatography, said method comprising:

routing fluid in a first groove from an inner end to an outer end of a first spiral flow path;

routing said fluid in a second groove from said outer end of said first spiral flow path to an inner end of a third groove; and

routing fluid in said third groove from an inner end to an outer end of a second spiral flow path.

19. The method of Claim 18, wherein said second groove extends substantially linearly.

20. A countercurrent chromatography apparatus comprising:

a series of coupled plates;

groove means in said plates for routing fluid through a plurality of spiral flow paths.

21. The apparatus of Claim 20, wherein said groove means is provided on first and second sides of at least one of said plates.

22. A method of performing sample separation comprising:

introducing a sample into a a column assembly, wherein the column assembly comprises a plurality of coupled separation disks, and wherein each of said separation disks comprises at least two interleaved spiral flow channels; and rotating said column assembly.

23. The method of Claim 22, wherein sad rotating comprises rotating in a synchronous planetary rotation.

24. The method of Claim 22, wherein sad rotating comprises mounting said column assembly on a rotating horizontal shaft.

SEPARATION DISK ASSEMBLY

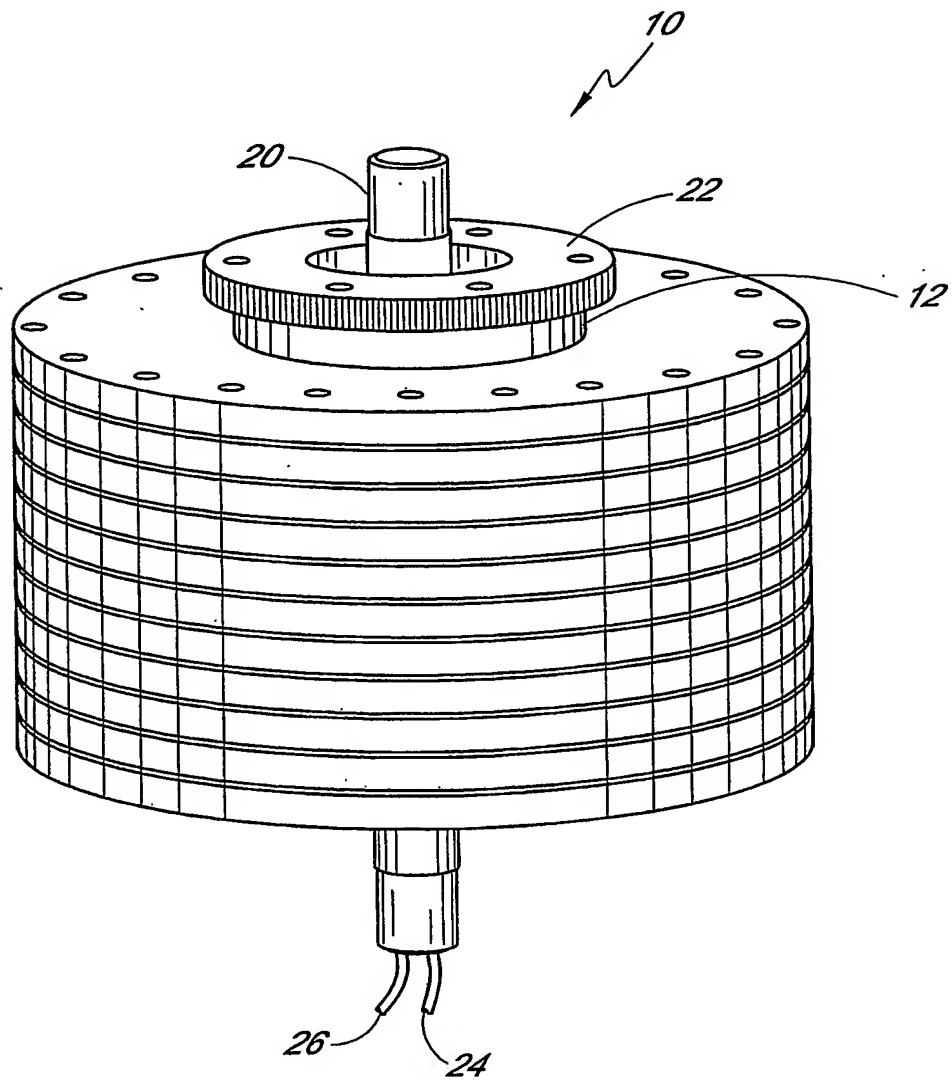


FIG. 1

COMPONENT OF SEPARATION DISK ASSEMBLY

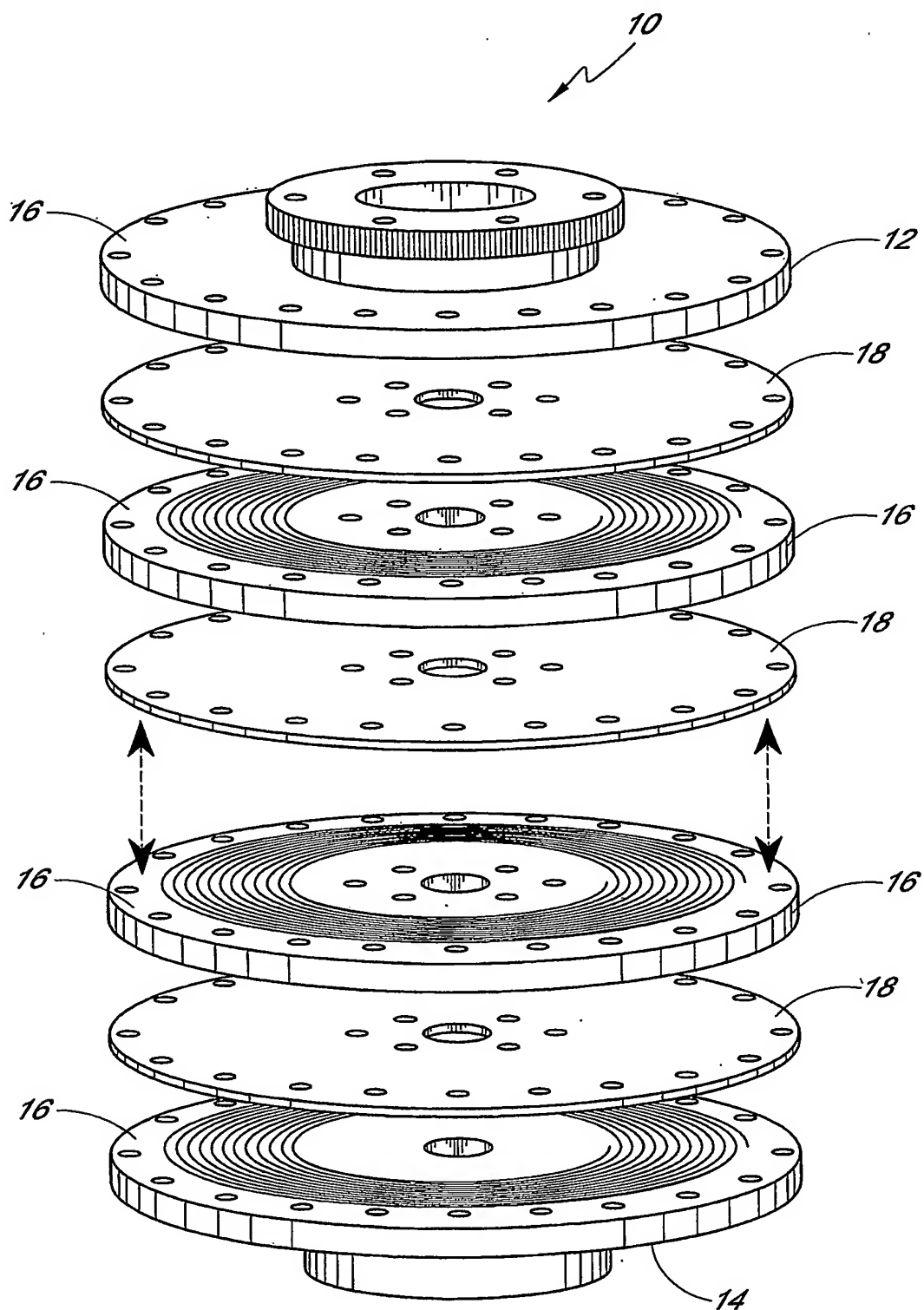


FIG. 2

SEPARATION DISK WITH SINGLE SPIRAL CHANNEL

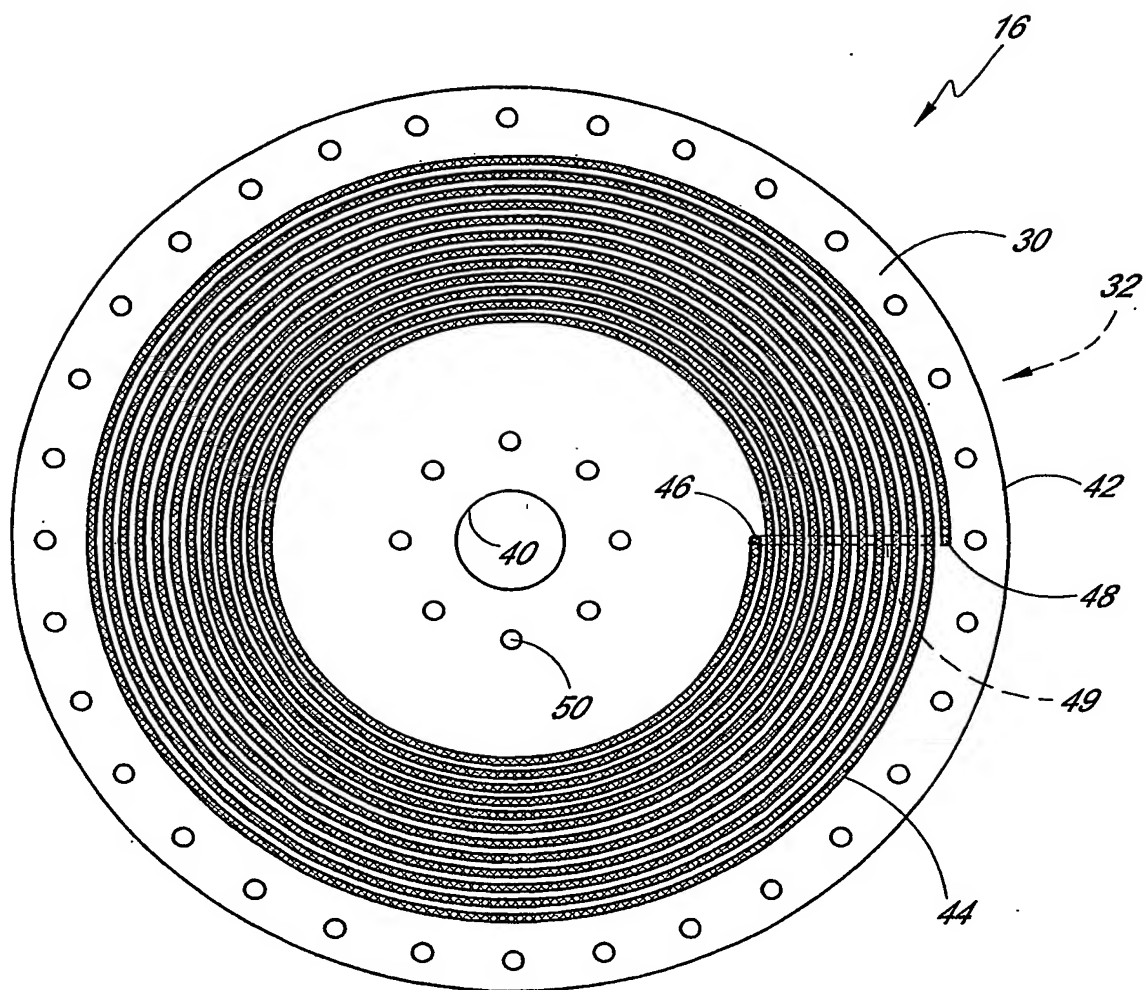


FIG. 3

SEPARATION DISK WITH 4 SPIRAL CHANNELS

FIG. 4A

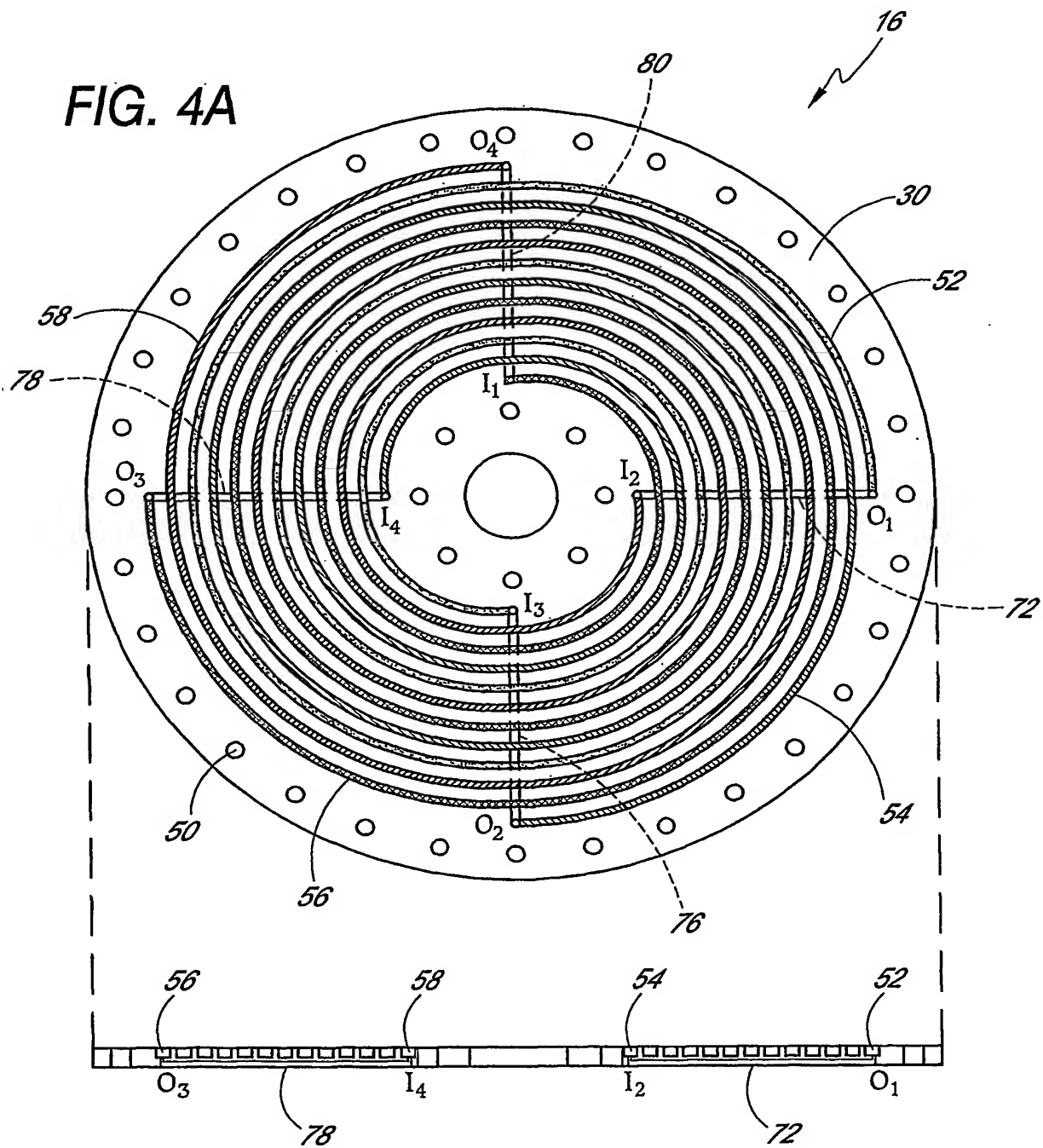


FIG. 4B

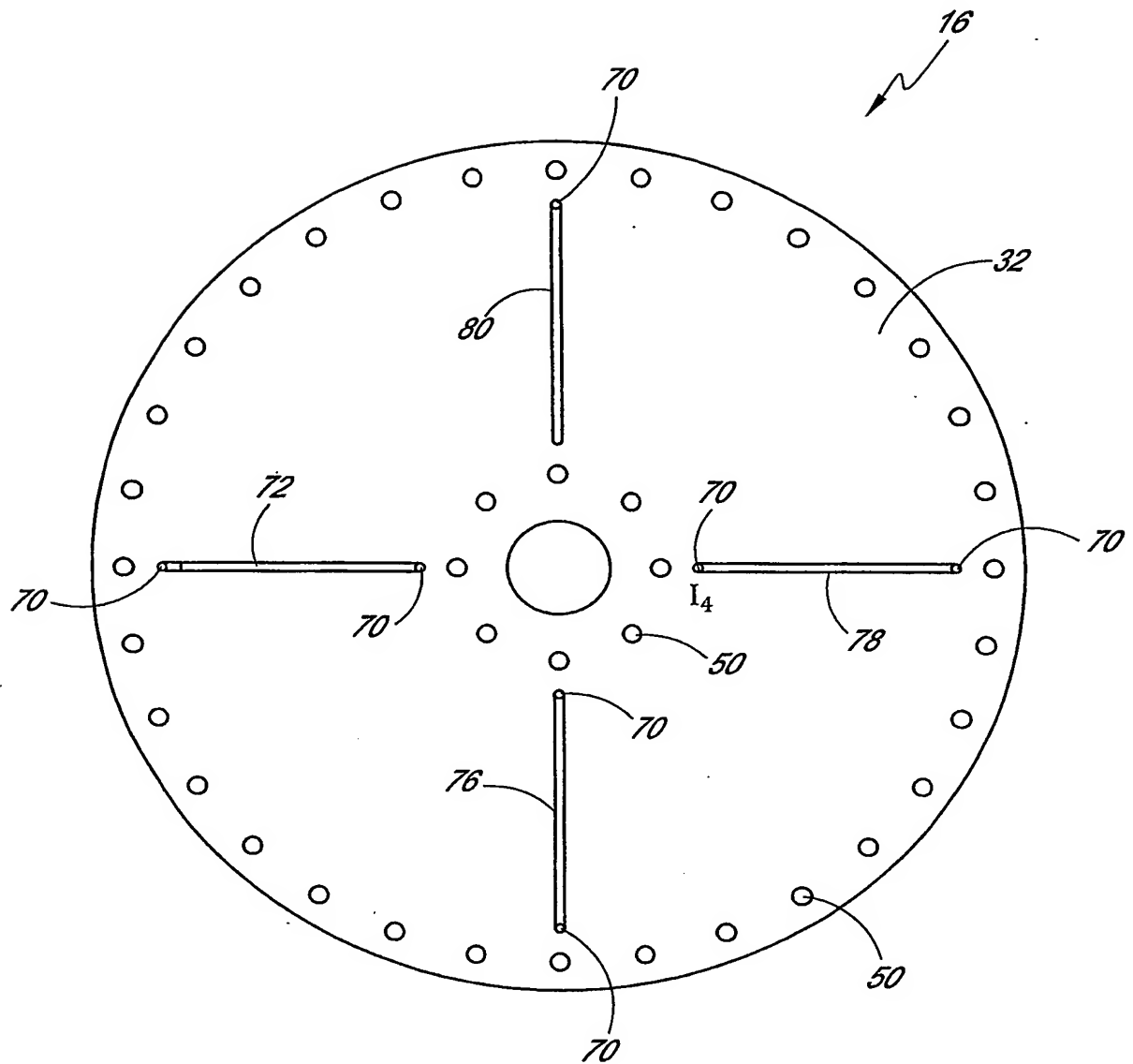


FIG. 4C

TEFLON SHEET SEPTUM

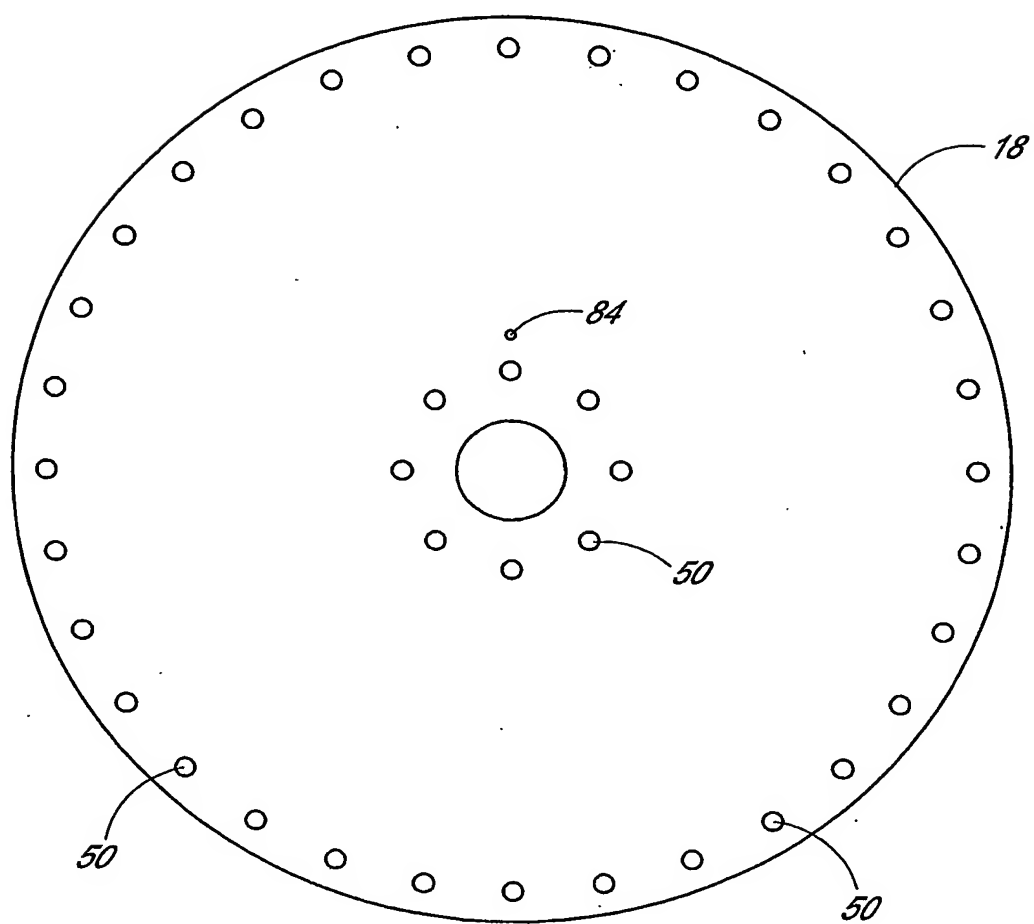
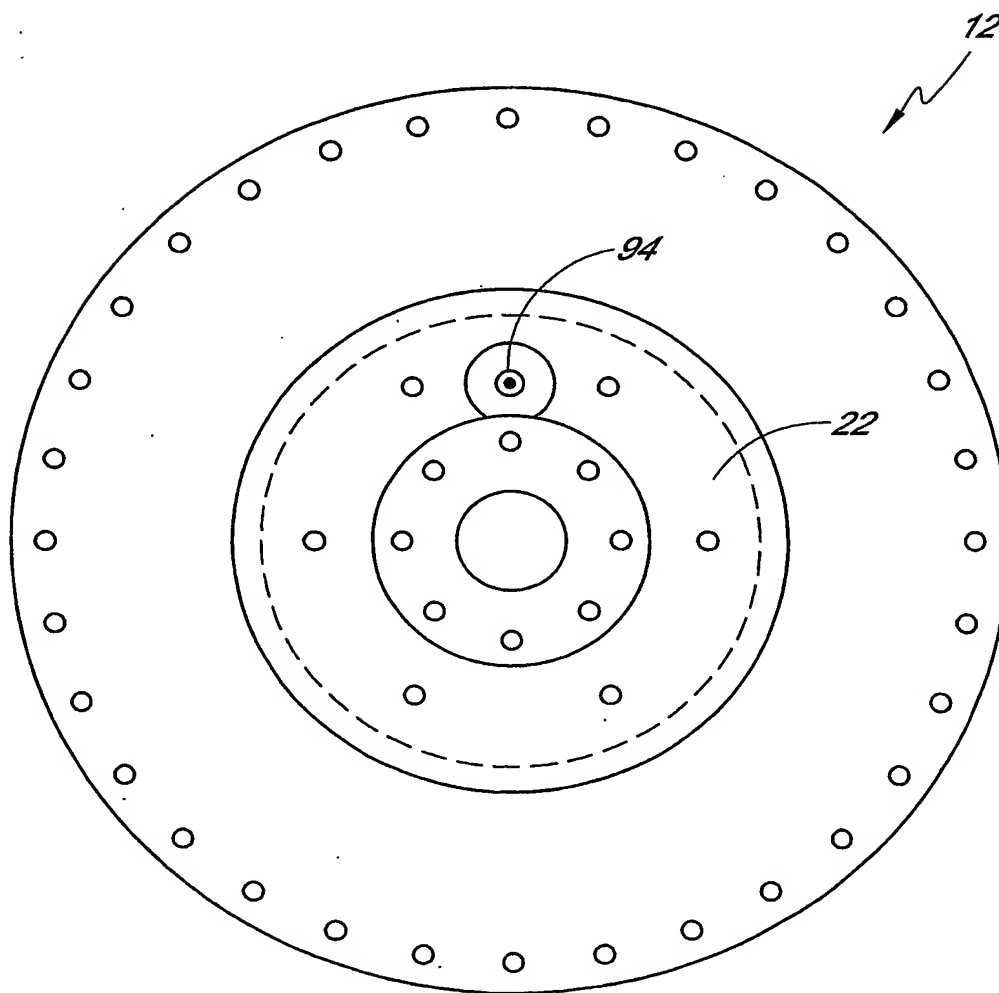
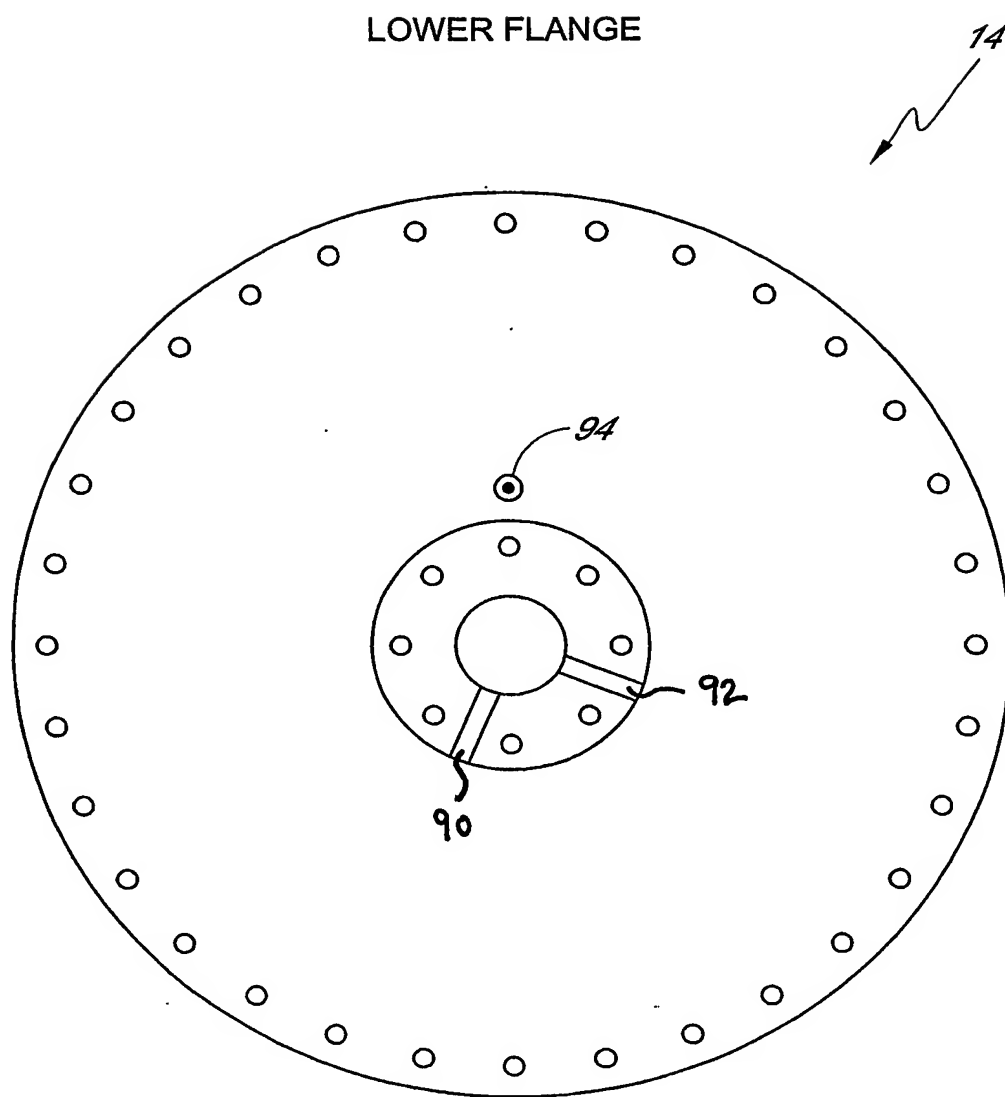


FIG. 5

UPPER FLANGE WITH A PLASTIC GEAR

**FIG. 6**

**FIG. 7**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/09189

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 30/02

US CL : 436/161; 422/70; 73/61.52, 61.53, 61.56; 210/656, 657, 198.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/161; 422/70; 73/61.52, 61.53, 61.56; 210/656, 657, 198.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 61-288154 A (YOKOGAWA ELECTRIC CORP.) 18 December 1986 (18.12.1986), Figures 1a-b and abstract.	20-21
Y	US 4,551,251 (KOLOBOW et al) 05 November 1985 (05.11.1985), Figure 7.	13, 18-21
Y	US 4,414,108 (ITO) 08 November 1983 (08.11.1983), Figures 9-10.	1-24
Y	US 4,968,428 (NUNOGAKI) 06 November 1990 (06.11.1990), Figures 11-12, column 9, lines 12-27.	1-24

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

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"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

29 May 2003 (29.05.2003)

Date of mailing of the international search report

16 JUN 2003

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks

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INTERNATIONAL SEARCH REPORT

PCT/US03/09189

Continuation of B. FIELDS SEARCHED Item 3:

EAST search terms: counter adj current, countercurrent, chromatogra\$8, spiral\$2, groove\$1, channel\$1, plate\$1, disc\$1, disk\$1, substrate\$1